Dietary Fat and Protein Interactions in the Broiler[1]

R. W. ROSEBROUGH,*,[2] J. P. MCMURTRY,* and R. VASILATOS-YOUNKEN†

Growth Biology Laboratory, Livestock and Poultry Science Institute, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, and †Department of Poultry Science, The Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT An experiment was conducted to study the interrelationships between dietary fat and protein levels in the regulation of lipid metabolism in the broiler chicken. Birds were fed diets containing 300, 600, or 1,200 kcal ME from fat (corn oil) with either 124 or 190 g CP/kg. Two additional experimental diets contained 234 or 285 g CP and 300 kcal ME from fat. Regardless of fat level, birds fed the diets containing 124 g CP/kg weighed less and were less efficient than birds fed diets containing 190 g CP/kg. The diet containing 600 kcal as fat decreased lipogenesis and malic enzyme activity ($P < 0.05$) in birds fed the diet containing 190 g CP/kg diet, but not in birds fed the diet containing 124 g CP/kg. Birds fed the latter level of protein required at least 1,200 kcal as fat to express any significant decrease in lipogenesis or malic enzyme activity ($P < 0.05$). Dietary fat did not affect plasma levels of triiodothyronine (T_3), thyroxine (T_4), or insulin-like growth factor-I (IGF-I). Feeding diets containing 124 g CP/kg resulted in decreased plasma T_4 and IGF-I and elevated T_3 ($P < 0.05$). Increasing dietary protein (compared to increasing dietary fat) increased body weights, IGF-I, T_4 and decreased lipogenesis, malic enzyme activity, and T_3. Both of these regimens involve decreasing dietary carbohydrate at equal rates, but results differed. Although replacement of dietary carbohydrates with either fat or protein reduce precursors for fat synthesis, both energy sources have additional unique effects on metabolism. Dietary protein levels modulate metabolic effects of dietary fat.

(*Key words*: dietary protein, dietary fat, lipogenesis, plasma hormones)

1999 Poultry Science 78:992–998

INTRODUCTION

It has been reported that dietary fat levels can influence lipid metabolism in birds. Balnave and Pierce (1969) reported that dietary fat decreased the *in vitro* incorporation of acetate into hepatic fatty acids. Yeh and Leveille (1971) fed large amounts of fat to chickens and noted a decrease in the quantity of free coenzyme A available to support *de novo* lipogenesis. This group speculated that decreased coenzyme A would decrease the catalytic potential of both acetyl coenzyme A and fatty acid synthetase by downregulation of both enzymes via long chain fatty acyl coenzyme A derivatives. It was generally thought that the inhibition of lipogenesis by dietary fat involved the flux rate of fatty acyl coenzyme A or availability of lipid precursors in the form of dietary carbohydrates. The latter hypothesis seemed reasonable because dietary fat was substituted for carbohydrate in most of the experiments.

We are unaware of any literature that describes the interaction of dietary fat and protein level in the regulation of lipogenesis. It is questionable whether either carbohydrate or fat intakes play a role in regulating lipogenesis in birds fed at a suboptimal plane of protein nutrition. It was of interest to determine whether the high rate of lipogenesis resulting from low protein diets (Rosebrough and Steele, 1985) could be attenuated by additional dietary fat. Knowledge about the role of dietary ingredients in this type of feeding regimen is lacking.

Received for publication August 31, 1998.

Accepted for publication February 27, 1999.

[1]Mention of a trade name, proprietary product or specific equipment is not a guarantee or a warranty by the USDA and does not imply an approval to the exclusion of other products.

[2]To whom correspondence should be addressed: Rosebro@LPSI.BARC.USDA.GOV

Abbreviation Key: AAT = aspartate aminotransferase; CHO = carbohydrate; GH = growth hormone; ICD-NADP = isocitrate, NADP+ oxidoreductase-[decarboxylating]; IGF-I = insulin like growth factor I; IVL = *in vitro* lipogenesis; MDH-NADP = malate, NADP+ oxidoreductase-[decarboxylating]; NADP = nicotinamide adenine dinucleotide phosphate; RIA = radioimmunoassay; T_3 = triiodothyronine; T_4 = thyroxine.

MATERIALS AND METHODS

Animals and Diets

All chickens were held under a quarantine that was certified by the station veterinarian. Chickens were observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Animal Use Committee.

At 7 d of age, male, Indian River broiler chickens were assigned to one of eight dietary treatments. Fat or protein energy was substituted for the carbohydrate energy in a basal diet (Table 1). The net results were diets containing either two levels of CP (120 or 190 g/kg) and either 300, 600, or 1,200 kcal ME from fat. Two additional experimental diets contained either 234 or 285 g CP/kg and 300 kcal ME from fat. The energy contributions of fat and CP were calculated as 9.0 and 4.0 ME/g, respectively. The remainder of the energy in each diet was assumed to be carbohydrate and was calculated by subtraction. The experiment was repeated for a total of eight pen replicates for each dietary treatment.

Housing and Sampling

The chickens were housed in battery brooders in an environmentally controlled room maintained at 23 C with a 12 h light-dark cycle (0600 to 1800 h light). Birds were given free access to feed and water. Treatments were systematically assigned to pens (four birds per pen) in each battery to balance positional effects across all treatments. The pens were 75 × 100 cm. In both experiments, chickens randomly were selected from each pen at 28 to 30 d of age, bled by cardiac puncture into blood collection tubes containing EDTA, and euthanatized by exsanguination. The blood samples were centrifuged at 600 × *g* and plasma samples were collected with individual Pasteur pipettes. Plasma samples were stored at –70 C for later analyses of hormones.

TABLE 1. Composition of the experimental diets

Ingredient	A	B	C	D	E	F	G	H
	(g/kg diet)							
Cornmeal	570	570	550	380	350	450	450	450
Soybean meal	140	90	80	80	75	200	40	180
Corn oil	10	45	115	17	52	115	15	15
Glucose	201	130	0	335	285	54	220	115
Isolated soy protein[1]	0	30	35	120	125	47	190	170
Dicalcium phosphate	40	40	40	40	40	40	40	40
Limestone	10	10	10	10	10	10	10	10
L-methionine[2]	5	5	5	5	5	5	5	5
Iodized salt	3	3	3	3	3	3	3	3
Selenium premix[3]	1	1	1	1	1	1	1	1
Mineral premix[4]	1	1	1	1	1	1	1	1
Vitamin premix[5]	5	1	1	1	1	1	1	1
Sand	5	40	75	0	20	23	10	5
Cellulose	9	30	80	0	22	40	10	0
Calculated composition								
Crude protein	124	124	124	190	190	190	234	285
Total ME, kcal/kg	3,194	3,215	3,219	3,457	3,467	3,470	3,345	3,193
Fat,[6] kcal/kg	300	603	1,210	297	598	1,207	297	305
Protein,[6] kcal/kg	496	596	496	763	760	760	936	1,140
Carbohydrate,[6] kcal/kg	2,400	2,108	1,515	2,400	2,108	1,503	2,109	1,746
Methionine	6.1	6.0	5.9	6.2	6.2	6.6	6.7	7.4
Cystine	1.6	1.5	1.4	1.6	1.5	2.1	1.7	2.5
Lysine	6.0	6.1	6.2	12.1	12.1	11.9	12.8	15.8
Tryptophan	1.3	1.2	1.2	1.7	1.7	1.9	2.1	2.7
Calcium[7]	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4
Phosphorus[7]	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2

[1]Soybean protein grade II (900 g/kg, 21726); Nutritional Biochemicals, Cleveland, OH 44122.

[2]L-methionine (18915), U.S. Biochemicals, Cleveland, OH 44122.

[3]Provided 0.2 mg Se/kg of diet.

[4]Provided (in milligrams per kilogram of diet), manganese, 100; iron, 100; copper, 10; cobalt, 1; iodine, 1; zinc, 100; and calcium, 89.

[5]Provided (in milligrams per kilogram of diet), retinol, 3.6; cholecalciferol, 0.075; biotin, 1; vitamin E, 10; riboflavin, 10; pantothenic acid, 20; choline, 2 g; niacin, 100; thiamine, 10; vitamin B_6, 10; menadione sodium bisulfite, 1.5; cyanocobalamin, 0.1; folic acid, 2; and ethoxyquin, 150.

[6]Fat was assumed to have 9.0 kcal ME/g; protein was assumed to have 4.0 kcal ME/g; energy from carbohydrate was derived by subtracting the derived energy values for fat and protein form the total energy value of each diet. Values for fat and protein were derived by multiplying these respective values by the calculated contributions of each ingredient.

[7]Calculated from the contribution of limestone and dicalcium phosphate only.

In Vitro *Metabolism*

Lipogenesis. Livers were excised, washed in 155 m*M* NaCl to remove exterior blood and debris. Pieces of fresh livers were sliced with a tissue chopper[3] at a setting corresponding to a thickness of 0.3 mm to give explants weighing from 35 to 75 mg. Quadruplicate explants were incubated at 37 C for 2 h in 3 mL of Hanks balanced salts (Hanks and Wallace, 1949; Rosebrough and Steele, 1987) containing 10 m*M* HEPES and 10 m*M* sodium[2-^{14}C]acetate (166 MBq/mol) under a 95% O_2-5% CO_2 atmosphere. At the end of the stated incubation periods, lipids in the explants were extracted with 10 mL of 2:1 chloroform:methanol for 18 h as described by Folch *et al.* (1957). The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts was measured by liquid scintillation spectroscopy. Activity for *in vitro* lipogenesis (IVL) was expressed as micromoles of acetate incorporated into total lipid per gram of tissue.

Enzyme Assays. Remaining liver tissues were homogenized (1,10, wt/vol) in 100 m*M* HEPES (pH 7.5)-3.3 m*M* β-mercaptoethanol and centrifuged at 12,000 *g* for 30 min (Rosebrough and Steele, 1985). The supernatant fractions were kept at –80 C until analyzed for the activities of malate, nicotinamide adenine dinucleotide phosphate oxidoreductase-[decarboxylating] (MDH-NADP), isocitrate, nicotinamide adenine dinucleotide phosphate oxidoreductase [decarboxylating] (ICD-NADP), and aspartate aminotransferase (AAT). The activity of MDH-NADP was monitored because of the enzyme's role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. In addition, ICD-NADP may function as both a residual source for the provision of NADPH and as a source of a coreactant for transamination. Finally, AAT aids in the removal of excess amine groups formed by feeding high protein diets.

The activity of MDH-NADP was determined by a modification of the method of Hsu and Lardy (1969). Reactions contained 50 m*M* HEPES (pH 7.5), 1 m*M* NADP, 10 m*M* $MgCl_2$ and the substrate, 2.2 m*M* L-malate (disodium salt) in a total volume of 1 mL. Portions (50 μL) of the 12,000 $\times$ *g* supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 C.

The activity of ICD-NADP activity was determined by a modification of the method of Cleland *et al.* (1969). Reactions contained 50 m*M*-HEPES (pH 7.5), 1 m*M* NADP, 10 m*M* $MgCl_2$ and the substrate, 4.4 m*M* DL-isocitrate in a total volume of 1 mL. Portions (50 μL) of the 12,000 $\times$ *g* supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 C.

The activity of AAT was determined by a modification of the method of Martin and Herbein (1976). Reactions contained 50 m*M* HEPES, 200 m*M* L-aspartate, 0.2 m*M* NADH, 1,000 units per liter malate, NAD+ oxidoreductase (EC 1.1.1.37) and the substrate, 15 m*M* 2-oxoglutarate in a total volume of 1 mL. Portions (25 μL) of the 12,000 $\times$ *g* supernatants (diluted 1:10) were preincubated in the presence of the first four ingredients. Reactions were initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 30 C. Enzyme activities are expressed as micromoles of product formed per minute under the assay conditions (Rosebrough and Steele, 1985).

Plasma Hormone Assays

Both triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured using a solid-phase single antibody procedure that is commercially available.[4] These assays were validated for avian samples (Rosebrough *et al.*, 1988) by dispersing standards in charcoal-stripped chicken sera and by noting recovery of added T_3 and T_4 (98%). Plasma insulin-like growth factor-I (IGF-I) was measured by radioimmunoassay (RIA) as previously described (McMurtry *et al.*, 1994). All assays were conducted as single batches to remove interassay variation. Plasma growth hormone (GH) concentrations were measured with a homologous chicken GH RIA (Vasilatos-Younken, 1986), using pituitary-derived chicken GH (cGH, Lot RVY03) as a standard and for iodination, and a rabbit anti-cGH serum as primary antibody. The anti-cGH antibody was raised against recombinant cGH[5] and detects both the pituitary-derived and recombinant cGH preparations with equal affinity as determined by competitive displacement curves. All hormone concentrations are reported as 10^{-9} *M*.

Statistical Analysis

Each particular combination of energy and CP was replicated eight times. A replicate was considered to be the pen of chickens treated alike (four chickens per pen). Dietary treatments were randomly allocated to chicken starter batteries to minimize positional effects (pens in batteries and batteries within the assigned room). The model used to analyze data was a one-way analysis of variance with each combination of fat and CP considered as a single treatment for a total of eight treatments. The replicate effect was considered as the positional effect and all treatments appeared at each position (battery and battery position). If the replicate effect was not found to be significant, this component of variation was pooled with the previous residual to provide an estimate of the pooled standard error. If the overall *F* test revealed significance of treatments, final comparisons of means were accomplished with Tukey's multiple range statistic using the

[3]MacIlwain Tissue Chopper, Mickle Laboratory Engineering Co., Gomshall, Surrey, U.K.
[4]ICN Biomedicals, Irvine, CA, 92602
[5]Lucky Biotech Corp., Emeryville, CA 94608.

TABLE 2. Dietary fat and protein energy substitutions for CHO on broiler chickens growing from 7 to 28 d of age[1,2]

Diet	Fat[3]	Carbohydrate	Protein[3]	Protein[4]	Body weight	Feed intake	Gain:feed
	(kcal/kg)				(g)		(g:g)
A	300	2,400	500	124	681[c]	1,583[a]	0.35[c]
B	600	2,100	500	124	717[c]	1,609[a]	0.36[c]
C	1,200	1,500	500	124	730[c]	1,716[a]	0.35[c]
D	300	2,400	760	190	1,170[b]	1,849[a]	0.56[b]
E	600	2,100	760	190	1,164[b]	1,873[a]	0.55[b]
F	1,200	1,500	760	190	1,147[b]	1,894[a]	0.54[b]
G	300	2,100	940	234	1,371[a]	1,874[a]	0.67[a]
H	300	1,750	1,140	285	1,175[b]	1,793[a]	0.59[ab]
Pooled SE					39	147	0.03

[a–c]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Mean values for eight observations per dietary treatment.

[2]Seven-day-old male, Indian River broiler chickens (average weight of 145 to 155 g) were fed diets containing 300, 600, or 1,200 kcal of energy as fat (124 or 190 g) or 300 kcal of energy as fat and 234 or 285 g/kg diet for a 21-d growth period. Chickens were then selected from each treatment at 28 d of age to determine the effects of dietary treatments on intermediary metabolism.

[3]Kilocalories per kilogram of diet (calculated from all feed ingredients). These values are rounded slightly for purposes of clarity. For actual, calculated values, see Table 1.

[4]Grams per kilogram of diet (calculated from all feed ingredients).

pooled standard error. These techniques have been described by Remington and Schork (1970).

RESULTS

Table 2 summarizes the effects of different levels of dietary fat and CP on growth performance of broiler chickens. Replacing dietary carbohydrate calories with fat calories did not change body weight, feed intake, or the efficiency of feed utilization. In contrast, birds fed diets with 190 g CP/kg (Diets D, E, F) ate more feed and used that feed more efficiently ($P < 0.05$) than did birds fed diets containing 120 g CP/kg (Diets A, B, C). In addition, this table also summarizes the effects of replacing carbohydrate with CP. Replacing carbohydrate energy with CP increased body weight and improved feed utilization when CP was substituted to give 2,100 kcal of carbohydrate energy (Diet G), but not when substituted to give 1,750 kcal of energy (Diet H).

Although dietary CP levels affected IVL (Diet H < Diet G <Diet D < Diet A), 600 kcal of dietary fat failed to suppress IVL in birds fed the lower level of CP (Diet B vs Diet A) but did suppress IVL in birds fed the higher level of (Diet D vs Diet E) (Table 3). A similar observation was noted for ME activity. In contrast, dietary fat levels did not influence the activities of either ICD-NADP or AAT. Isocaloric substitution of dietary carbohydrate with CP (Diet G and H) decreased IVL, an effect that contrasts with the above observation. Malic enzyme activities followed these same trends. The activity of AAT was increased by the higher rate of CP for carbohydrate substitution (Diet H).

Table 4 shows that dietary fat levels had little influence on plasma IGF-I, T_3, and T_4. Plasma IGF-I and T_4 were greater ($P < 0.05$) and T_3 less ($P < 0.05$) in birds fed the higher level of CP. Substitution of CP, but not fat, for carbohydrate increased plasma IGF-I and T_4 ($P < 0.05$). In contrast, this same substitution regimen (CP for carbohydrate) decreased plasma GH. The substitution of CP for carbohydrate resulting in a CP content of 285 g and 1,140 kcal/kg gave the only consistent decrease in plasma T_3.

DISCUSSION

The data in the present study indicate that the level of dietary CP must be considered when dietary fat is used to decrease *de novo* lipogenesis. Furthermore, when dietary carbohydrate energy was lowered and replaced with either fat or CP energy, far different results were noted. Dietary fat additions to diets containing low CP levels did not decrease lipogenesis to the degree noted when added to a diet containing a higher level of CP. In this respect, it could be hypothesized that carbohydrate availability may not regulate lipid metabolism in birds fed diets containing very low levels of CP. Yeh and Leveille (1969) found an inverse relationship between the level of dietary CP and the subsequent rate of IVL, and speculated that an increase in the dietary CP level decreased the flow of substrates through glycolysis and increased the production of glucose from substrates that were formerly in the pathways leading to fat synthesis. Based on a high correlation between ME activity and *de novo* lipogenesis, Yeh and Leveille (1969) originally proposed that availability of NADPH regulates lipid metabolism in chickens fed high protein diets.

Allman and Gibson (1969) and Clarke *et al.* (1976) proposed specific effects of fatty acids, based on their degree of polyunsaturation. For example, 20 g of linolenic acid/kg of diet depressed *de novo* lipogenesis in

TABLE 3. Dietary fat and protein energy substitutions for carbohydrate *in vitro* metabolism in broiler chickens growing from 7 to 28 d of age[1,2]

Diet	Fat[3]	Carbohydrate	Protein[3]	Protein[4]	IVL[5]	MDH-NADP[6]	ICD-NADP[6]	AAT[6]
	(kcal/kg)			(g/kg)				
A	300	2,400	500	124	45.8[a]	22.7[a]	22.9[ab]	44.8[b]
B	600	2,100	500	124	44.7[a]	17.6[b]	23.1[ab]	46.5[b]
C	1,200	1,500	500	124	30.4[b]	15.2[ab]	27.3[ab]	53.7[b]
D	300	2,400	760	190	35.1[b]	17.6[b]	21.7[ab]	50.8[b]
E	600	2,100	760	190	21.7[c]	14.6[ab]	19.5[b]	42.2[b]
F	1,200	1,500	760	190	15.6[cd]	12.9[c]	23.3[ab]	44.5[b]
G	300	2,100	940	234	19.1[c]	11.7[c]	35.0[a]	47.7[b]
H	300	1,750	1,140	285	10.2[d]	7.3[d]	34.2[a]	66.9[a]
Pooled SE					2.1	1.1	3.5	3.1

[a–d]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Mean values for eight observations per dietary treatment.

[2]For a description of dietary treatments, see Table 2.

[3]Kilocalories per kilogram of diet (calculated from all feed ingredients). These values are rounded slightly for purposes of clarity. For actual, calculated values, see Table 1.

[4]Grams per kilogram of diet (calculated from all feed ingredients).

[5]*In vitro* lipogenesis (IVL) was determined by culturing liver explants for 2 h in the presence of 10 m*M* [2-^{14}C]sodium acetate and by noting incorporation of acetate into hepatic lipids. Values are expressed as micromoles substrate incorporated per gram of liver.

[6]Enzyme activity is noted as micromoles of oxidized or reduced NAD(P) per minute per gram of liver at 30 C. AAT = aspartate aminotransferase.

the mouse. A decrease in the activities of several enzymes involved in lipogenesis also accompanied the feeding of this fatty acid. This initial report was followed by additional studies showing that neither saturated nor monounsaturated fatty acids specifically changed lipogenesis (Clarke *et al.*, 1976; Clarke and Clarke, 1982; Blake and Clarke, 1990; Dax *et al.*, 1990; Armstrong *et al.*, 1991; Clarke and Jump, 1993, 1994; DaSilva *et al.*, 1993).

Certain studies in the past have examined dietary energy influences on plasma thyroid hormones. For example, Schalch and Cree (1985) found that reducing fat calories decreased body weight and T_3 and T_4 in young rats. A later report from this same group (Yang *et al.*, 1987) indicated that reducing carbohydrate energy also decrease body weight, but did not change either T_3 or T_4. The data in the present study indicate that plasma T_3 and T_4 concentrations reflect the state of protein nutriture of the chicken. Generally speaking, we have found that plasma thyroid hormones will respond to changes in diet that elicit large changes in growth (Rosebrough *et al.*, 1988; Rosebrough and McMurtry,

TABLE 4. Dietary fat and protein energy substitutions for carbohydrate on plasma hormone concentrations in broiler chickens growing from 7 to 28 d of age[1,2]

Diet	Fat[3]	Carbohydrate	Protein[3]	Protein[4]	IGF-I	GH	T_3	T_4
	(kcal/kg)			(g/kg)				
A	300	2,400	500	124	2.05[c]	1.36[a]	5.27[a]	8.59[d]
B	600	2,100	500	124	2.17[c]	1.53[a]	5.28[a]	8.81[d]
C	1,200	1,500	500	124	2.80[c]	1.34[a]	4.87[ab]	8.83[d]
D	300	2,400	760	190	3.51[b]	0.83[b]	4.39[b]	15.78[ab]
E	600	2,100	760	190	3.89[b]	0.82[b]	4.50[b]	13.62[c]
F	1,200	1,500	760	190	3.89[b]	0.80[b]	4.35[b]	13.55[c]
G	300	2,100	940	234	3.97[ab]	0.86[b]	2.82[c]	16.53[b]
H	300	1,750	1,140	285	4.56[a]	0.75[b]	2.41[c]	20.68[a]
Pooled SE					0.15	0.06	0.17	0.58

[a–d]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Mean values for eight observations per dietary treatment. IGF-I = insulin like growth factor-I; GH = growth hormone; T_3 triiodothyronine; T_4 = thyroxine.

[2]For a description of dietary treatments, see Table 2.

[3]Kilocalories per kilogram of diet (calculated from all feed ingredients). These values are rounded slightly for purposes of clarity. For actual, calculated values, see Table 1.

[4]Grams per kilogram of diet (calculated from all feed ingredients).

1998). In contrast, using thyroid hormones as indicators of changes in nutritional status during a production environment may be difficult because of subtle differences in diets that are not found in certain experimental diets (Rosebrough *et al*., 1992). In fairness, this criticism can be applied to the use of any hormone as an indicator of protein nutriture.

In comparing plasma IGF-I concentrations among animal species, lower concentrations were noted in the chicken (Leung *et al*., 1986) than in the growing rat (Prewitt *et al*., 1982), although trends associated with growth are similar in both species. Growth retardation in chickens may be due also to reduced systemic IGF-I as well as increased catabolic hormones. Buyse *et al*. (1986) used exogenous corticosterone to reduce growth and increase body fat of chickens and found a decrease in plasma IGF-I concentration. Huybrechts *et al*. (1985) surveyed plasma IGF-I concentrations in growing meat- and egg-type chickens and found an age-dependent decrease in plasma IGF-I in the layer but not in the broiler chicken. The latter observation is not surprising because the meat-type chicken has been intensely selected for rapid growth.

Lauterio and Scanes (1987) reported a decrease in plasma IGF-I when chicks were switched from a diet containing 200 to one containing 50 g of CP/kg. Correspondingly, the opposite feeding regimen increased plasma IGF-I. A later study (Rosebrough *et al*., 1988) determined that plasma IGF-I concentrations matched both growth and relative breast muscle size, which suggests that this hormone regulated lean tissue development.

It should also be mentioned that in many of the above studies, plasma GH concentrations were either not related to growth or were related inversely The latter relationship casts some doubt on the putative GH-IGF-I axis, whereby GH induces IGF-I production. In fairness, it should be noted that GH is secreted in a pulsatile manner and that sampling intervals may have influenced values attained in these studies. The pulsatile secretion pattern was probably not the sole cause of GH values in the present study. If this was the case, all birds exhibiting low GH values would have to have been sampled at the nadir of the secretion profile. Likewise, all birds exhibiting higher GH values would have had to be sampled at the zenith of the secretion profile. Thus, if management criteria for the particular experiment can be controlled (lighting intervals, feeding times, etc.), single point values for GH can be used. Results reinforce the concept that, at times, there is no relationship between GH and IGF, which presents a conflict because of the putative control of IGF-I by GH (Roberts *et al*., 1986). A large body of work indicates that low GH levels are found in rapidly growing birds compared to more slowly growing birds (Burke and Marks, 1982; Stewart and Washburn, 1983). Although Leung *et al*. (1986) and Vasilatos-Younken *et al*. (1988a,b) increased body weights of chickens with exogenous GH, natural levels of GH have a relationship to growth that is dissimilar to levels attained by artificial means.

REFERENCES

Allman, D. W., and D. W Gibson, 1969. Fatty acid synthesis during early linoleic acid deficiency in the mouse. J. Lipid Res. 6:51–62.

Armstrong, M. K., W. L. Blake, and S. D. Clarke, 1991. Arachidonic acid suppression o fatty acid synthase gene expression in cultured rat hepatocytes. Biochem. Biophys. Res. Comm. 177:1056–1061.

Balnave, D., and J. Pierce, 1969. Adaptation of laying hens (Gallus domesticus) to dietary fat with special reference to changes in liver and ovarian lipid content and liver enzyme activity. Comp. Biochem. Physiol. 29:539–550.

Blake, W. L., and S. D. Clarke, 1990. Suppression of rat hepatic fatty acid synthase and $S1_4$ gene transcription by dietary polyunsaturated fat. J. Nutr. 120:1727–1729.

Burke, W. H., and H. L. Marks, 1982. Growth hormone and prolactin levels in non-selected and selected broiler lines of chickens from hatch to eight weeks of age. Growth 46: 283–295.

Buyse, J., E. Decuypere, L. M. Huybrechts, E. R. Kuhn, and C. Whitehead, 1986. Effect of corticosterone on circulating concentrations of corticosterone, prolactin, thyroid hormones and somatomedin-C and on fattening in broilers selected for high or low fat content. J. Endocrinol. 112: 229–237.

Clarke, B. A., and S. D. Clarke, 1982. Suppression of rat liver fatty acid synthesis by eicosa-5,8,11,14-tetraynoic acid without a reduction in lipogenic enzymes. J. Nutr. 112: 1212–1219.

Clarke, S. D., and D. B. Jump, 1993. Regulation of gene transcription by polyunsaturated fatty acids. Prog. Lipid Res. 32:139–149.

Clarke, S. D., and D. B. Jump, 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. Ann. Rev. Nutr. 14:83–98.

Clarke, S. D., D. R. Romsos, and G. A. Leveille, 1976. Specific inhibition of hepatic fatty acid synthesis exerted by dietary linoleate and linolenate in essential fatty acid adequate rats. Lipids 11:485–490.

Cleland, W. W., V. M. Thompson, and R. E. Barden, 1969. Isocitrate dehydrogenase (TPN specific) from pig heart. Pages 30–33 *in*: Methods in Enzymology. Vol. 13. J. M. Lowenstein, ed. Academic Press, New York, NY.

DaSilva, L. A., O. L. DeMarcucci, and Z. R. Kuhnle, 1993. Dietary polyunsaturated fats suppress the high-sucrose-induced increase of rat liver pyruvate dehydrogenase levels. Biochem. Biophys. Acta 1169:126–134.

Dax, E. M., J. S. Partilla, M. A. Pilleyo, and R. I. Gregerman, 1990. Altered glucagon and catecholamine hormone-sensitive adenyl cyclase responsiveness in rat liver membranes induced by manipulation of dietary fatty acid intake. Endocrinology 127:2236–2241.

Folch, J., M. Lees, and G. H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Hanks, J. H., and R. E. Wallace, 1949. Relation of oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. Exp. Biol. Med. 71:196–200.

Hsu, R. Y., and Lardy, H. A., 1969. Malic enzyme. Pages 230–235 *in*: Methods in Enzymology. Vol. 13. J. M. Lowenstein, ed. Academic Press, New York, NY.

Huybrechts, L. M., D. B. King, T. J. Lauterio, J. Marsh, and C. G. Scanes, 1985. Plasma concentrations of somatomedin-C in hypophysectomized, dwarf and intact domestic fowl as determined by heterologous radioimmunoassay. J. Endo. 104:233–239.

Lauterio, T. J., and C. G. Scanes, 1987. Hormonal responses to protein restriction in two strains of chickens with different growth characteristics. J. Nutr. 117:758–763.

Leung, F. C., J. E. Taylor, S. Wein, and A. Van Iderstine, 1986. Purified chicken growth hormone and a human pancreatic GH-releasing hormone increase body weight gain in chickens. Endocrinology 118:1961–1965.

Martin, R. J., and J. H. Herbein, 1976. A comparison of the enzyme levels and *in vitro* utilization of various substrates for lipogenesis in pair-fed lean and obese pigs. Proc. Soc. Exp Biol. Med. 151:231–235.

McMurtry, J. P., G. L. Francis, F. Z. Upton, G. Rosselot, and D. M. Brocht, 1994. Developmental changes in chicken and turkey insulin like growth factor I (IGF-I) studied with a homologous radioimmunoassay for chicken IGF-I. J. Endo. 142:225–234.

Prewitt, D. S., A. J. D'Ercole, and B. R. Switzer, 1982. Relationship of serum immunoreactive somatomedin-C to dietary protein and energy in growing rats. J. Nutr. 112: 144–150.

Remington, R. D., and M. A. Schork, 1970. Statistics with Applications to the Biological and Health Sciences Prentice-Hall, Englewood Cliffs, NJ.

Roberts, C. T., Jr., A. L. Brown, D. E. Grahm, and S. Berry, 1986. Growth hormone regulates the abundance of insulin-like growth factor-I RNA in adult rat liver. J. Biol. Chem. 261:10025–10028.

Rosebrough, R. W., and J. P. McMurtry, 1998. Further studies on carry over effects of dietary crude protein and triiodothyronine (T_3) in broiler chickens. Br. J. Nutr. 79: 89–85.

Rosebrough, R. W., J. P. McMurtry, A. D. Mitchell, and N. C. Steele, 1988. Protein and energy restrictions in the broiler chicken. 6. Effect of dietary protein and energy restrictions on carbohydrate and lipid metabolism and metabolic hormone profiles. Comp. Biochem. Physiol. 90:311–316.

Rosebrough, R. W., J. P. McMurtry, and R. Vasilatos-Younken, 1992. In vitro lipid metabolism, growth and metabolic hormone concentrations in hyperthyroid chickens. Br. J. Nutr. 68:667–676.

Rosebrough, R. W., and N. C. Steele, 1985. Energy and protein relations in the broiler 1. Effect of protein levels and feeding regimes on growth, body composition and *in vitro* lipogenesis in broiler chickens. Poultry Sci. 64:119–126.

Rosebrough, R. W., and N. C. Steele, 1987. Methods to assess glucose and lipid metabolism in avian liver explants. Comp. Biochem. Physiol. 88A:1041–1049.

Schalch, D. S., and T. C. Cree, 1985. Protein utilization in growth: Effect of lysine deficiency on serum growth hormone, somatomedins, insulin, total thyroxine and triiodothyronine, free T_4 index, and total corticosterone. Endocrinology 117:667–673.

Stewart, P. A., and K. W. Washburn, 1983. Variation in growth hormone, triiodothyronine (T_3) and lipogenic enzyme activity in broiler strains differing in growth and fatness. Growth 47:411–425.

Vasilatos-Younken, R., 1986. Preparation and culture of dispersed avian pituitary cells, and age-related changes in donor pituitary weight and growth hormone content. Gen. Comp. Endocrinol. 64:99–106.

Vasilatos-Younken, R., T. L. Cravener, M. G. Mast, L. A. Cogburn, and R. H. Wellenreiter, 1988a. Effect of chronic, pulsatile, intravenous administration of pituitary-derived chicken GH (cGH) on endocrine and metabolite parameters in broiler-strain pullets. Poultry Sci. 67(Suppl. 1):168. (Abstr.)

Vasilatos-Younken, R., T. L. Cravener, M. G. Mast, L. A. Cogburn, and R. H. Wellenreiter, 1988b. Effect of pattern of administration on the response to exogenous pituitary-derived chicken growth hormone by broiler-strain pullets. Gen. Comp. Endocrinol. 712:268–283.

Yang, H., T. C. Cree, and D. S. Schalch,, 1987. Effect of a carbohydrate-restricted, calorie-restricted diet on the growth of young rats and on serum growth hormone, somatomedins, total thyroxine and triiodothyronine, free T_4 index and corticosterone. Metabolism 26:794–798.

Yeh, Y. Y., and G. A. Leveille, 1969. Effect of dietary protein on hepatic lipogenesis in the growing chick. J. Nutr. 98: 356–366.

Yeh, Y. Y., and G. A. Leveille, 1971. Studies on the relationship between lipogenesis and the level of coenzyme A derivatives, lactate and pyruvate in the liver of the growing chick. J. Nutr. 101:911–920.